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Differential Induction of *Escherichia coli* Autolysis by Penicillin and the Bacteriophage ϕX174 Gene *E* Product

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The behavior of the temperature-sensitive, penicillin-tolerant *Escherichia coli* mutant VC44 to endogenously induced autolysis by the bacteriophage ϕX174 gene *E* product (gpE) was investigated. Expression of the cloned ϕX174 lysis gene showed that cultures of strain VC44 grown at the restricted temperature were fully sensitive to endogenously induced autolysis. The results revealed that the modes of *E. coli* lysis induction by gpE and by penicillin differ and that the trigger mechanisms for autolysis depend greatly on the specific inducer used.

Several investigations have aimed at a genetic definition of the signals which trigger (deregulate) the activity of the autolytic system in *Escherichia coli* (6, 13, 18). If such regulatory factors of the autolytic system exist, the tolerance phenotype of the mutated bacteria could be independent of the pathway of lysis induction. To test this assumption, one can use the controlled expression of viral lysis genes as activators of cell lysis-from-within in conjunction with bacterial mutants deficient in drug-induced lysis. Such genes have been cloned from the single-stranded DNA phage ϕX174 (3, 8, 19) and the single-stranded RNA phage MS2 (4, 10, 11).

Using the temperature-sensitive autolysis-defective *E. coli* mutant VC30, which has a genetic lesion at 58 min (*lytA*) on the *E. coli* linkage map (R. E. Harkness, Ph.D. thesis, University of Victoria, Victoria, British Columbia, Canada, 1984), it was shown that autolysis of *E. coli* by the gene *E* product (gpE) of ϕX174 and by penicillin share cellular prerequisites (13). Other factors, such as growth rate, pH of the culture medium, or osmotic-stabilizing agents, also influence both penicillin- and gpE-induced lysis (12, 13, 17).

Because of the lysis-negative phenotype of VC30 after treatment with penicillin or gpE under restricted conditions, it was not possible to determine clearly whether the mode of lysis induction is similar for both agents or whether a regulatory function of the autolytic cascade, common for unrelated triggering mechanisms, is impaired. The latter possibility seemed to be more likely, as treatment with D-cycloserine or deprivation of nutritionally required diaminopimelic acid was also tolerated by the mutant under restricted conditions (7).

In this study, the temperature-sensitive β-lactam-tolerant *E. coli* mutant VC44 (16) was used to elucidate this question. Cultures of strain VC44 infected with phage ϕX174 K9 (host range mutant; 5) lysed completely at the unrestricted temperature (30°C). Under conditions in which penicillin tolerance is expressed (30 min after temperature upshift to 42°C), infection with ϕX174 was followed by a slight decrease in culture density, leaving almost 75% of the bacteria intact (data not shown).

Because tolerance to penicillin by strain VC44 is first expressed after 30 min (16), it can be assumed that the portion of the culture which did not establish tolerance to phage-induced lysis would do so when incubated for a longer period at the restricted temperature. In fact, preincubation of the cells for 45, 50, or 60 min at 42°C before infection with ϕX174 rendered all cells tolerant to ϕX174-induced lysis (Fig. 1). Therefore, all the following investigations were performed with cells which were phage infected after 45 min of incubation at the restricted temperature.

Tolerance to penicillin-induced lysis in strain VC44 is reversible by a temperature downshift from 42 to 30°C. Under these conditions, cell lysis occurs even in the presence of protein synthesis inhibitors (16). Temperature downshift of phage-infected VC44 cells from the restrictive temperature in the presence of chloramphenicol or kanamycin was not followed by lysis (Fig. 2a and b).

Assaying intracellular phages at various times after infection indicated that the ϕX174-infected cells were impaired in phage replication at the restricted temperature. *E. coli* VC44 was infected with ϕX174 K9 (multiplicity of infection, 2.5) 45 min after a temperature upshift at 42°C (time zero). Samples (1 ml) were withdrawn at various times after infection, and intracellular progeny phages were determined as previously described (12). The titer at time zero, 0.1 PFU × 10^{-7}, was the background of reversible adsorbed phages under the conditions used. Phage titers were 1, 4.5, 8, and 9 PFU ×

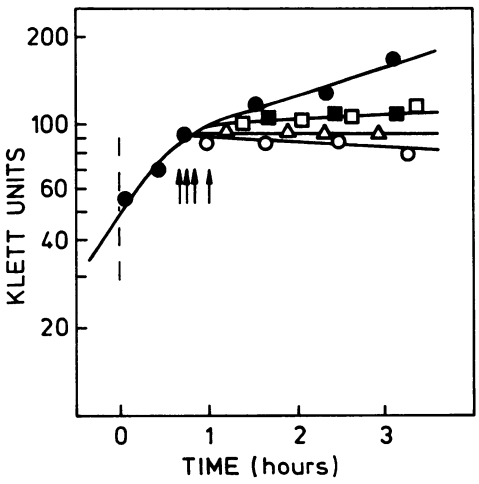

FIG. 1. ϕX174 infection of *E. coli* VC44 after prolonged growth at 42°C. At time 0, an exponentially growing culture of strain VC44 was shifted from 30 to 42°C (●). At the following times after temperature upshift (arrows), samples were infected with ϕX174 (multiplicity of infection, 2.5): 40 (○), 45 (△), 50 (□), and 60 (■) min.

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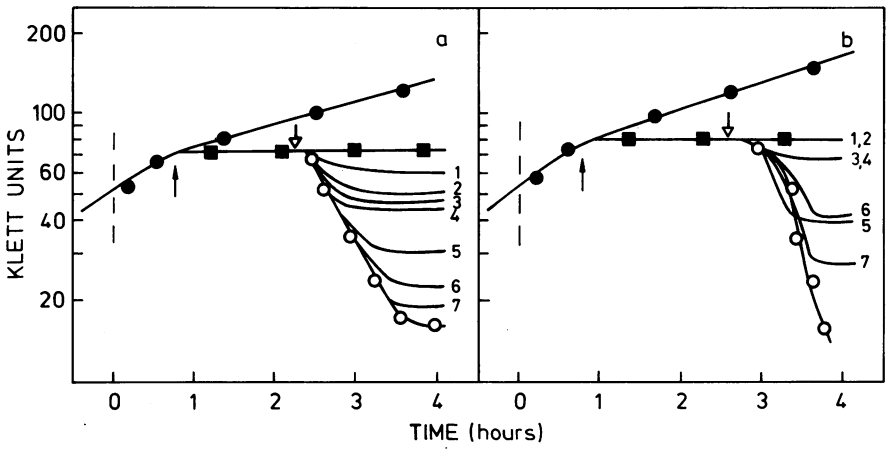

FIG. 2. Temperature downshift of φX174-infected cultures of *E. coli* VC44 from 42 to 30°C in the presence of protein synthesis inhibitors. (a) After 45 min of growth at 42°C (●), *E. coli* VC44 was infected with φX174 (multiplicity of infection, 2.5, closed arrow [■]). After 2 h at 42°C (open arrow), portions of the sample were treated with chloramphenicol (200 μg/ml) at various times after temperature downshift (○):1, 0 min; 2, 10 min; 3, 20 min; 4, 30 min; 5, 40 min; 6, 50 min; and 7, 60 min. (b) The same conditions were used as in panel a, but kanamycin (50 μg/ml) took the place of chloramphenicol.

10^{-7} at 20, 40, 60, and 120 min after infection, respectively. It was concluded that at the restricted temperature, owing to an unspecific effect of phage replication, gene *E* was not expressed adequately to induce the lysis of strain VC44.

To avoid complications arising from impaired phage replication, further experiments were performed with plasmid-encoded gene *E*. Plasmid pUH123, which was used for this purpose, carries gene *E* under the transcriptional control of *lacpo* (9). Gene *E* can be expressed from this plasmid by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to transformed cultures (8, 9). As can be seen in Fig. 3, strain VC44 was fully sensitive to gpE-induced lysis at both the permissive and nonpermissive temperatures. Because of the full sensitivity of strain VC44 under both conditions, it was not surprising that onset of lysis after the induction of gene *E* transcription was much more rapid at 42°C (Fig. 3b) than at 30°C (Fig. 3a). The rate of lysis also was accelerated at the higher temperature.

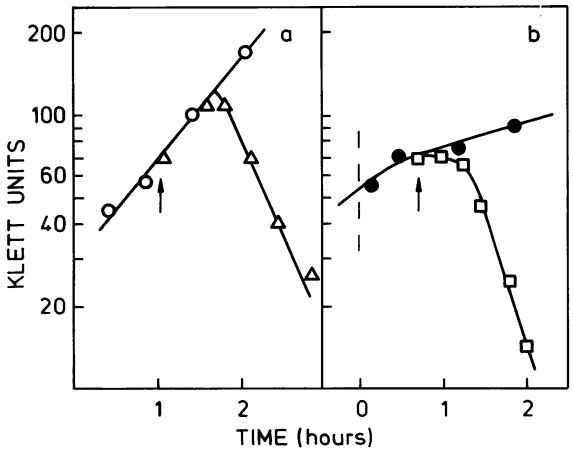

FIG. 3. Expression of cloned gene *E* in strain VC44. (a) IPTG was added to a sample of exponentially growing *E. coli* VC44(pUH123) at 30°C (△) at the time indicated by the arrow. Untreated control (○). (b) At the time indicated by the dashed line, an exponentially growing culture of strain VC44(pUH123) was shifted from 30 to 42°C (●); 45 min after temperature upshift (arrow), IPTG was added to a portion of the culture (□).

gpE-induced lysis of *E. coli* is a result of its integration into the host cell inner membrane (1, 2). In association with this membrane integration, a very limited degradation of peptidoglycan occurs (14). Hence, it is possible that gpE-induced autolysis of *E. coli* requires a specific interaction of the phage protein with enzymes which also represent targets for penicillin (17). On the other hand, a more indirect mechanism for the activation of peptidoglycan-synthesizing or -degrading enzymes as a consequence of membrane perturbation by gpE is also possible (15).

These findings indicate that the mutation conferring penicillin tolerance in strain VC44 does not affect an essential function for gpE-induced autolysis. Despite the fact that a variety of common features for penicillin and gpE action exist, our findings do not support a similar pathway for gpE- and penicillin-induced lysis. This conclusion is further supported by the ability of gpE to induce lysis in other mutants with a β-lactam-tolerant phenotype (6).

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